skite spectra (Fig. 3, B and C). The new peaks were sharp; often their widths were below the resolution limit of our Ge detector (~0.22 keV full width at half maximum). Such conditions are characteristic of reduced shear stresses (due to low sample strengths), and they suggest that the sample temperatures are higher than that associated with the orthorhombic-to-cubic transformation. The new peaks can be indexed as a mixture of (Mg,Fe)O (magnesiowüstite) and rutile-structured SiO_2 (stishovite). That is, our sample appears to have dissociated to mixed oxides and perovskite after prolonged laser heating at high temperatures. Although we cannot rule out the possibility of small amounts of partial melting during the formation of magnesiowüstite and stishovite, large-scale melting seems unlikely because of the strong intensity of the diffraction lines. The oxide lines remained after the sample was quenched, an indication that the transition is irreversible over the time scales of cooling in these experiments. Close inspection of the 211 line of stishovite shows a splitting on cooling, however (Fig. 3C). This distortion is consistent with the formation of the $CaCl_2$ phase at room temperature, as observed in diffraction and spectroscopic experiments at comparable pressures (*17*).

In general, our results are consistent with earlier diamond cell studies showing orthorhombic silicate perovskite as the primary phase in quench experiments after moderate laser heating at pressure ≥25 GPa for samples with comparable iron contents (*1*). Our data indicate that cubic silicate perovskite could have been synthesized in earlier experiments, but it would not have been observed because of the reversibility on quenching to room temperature conditions. Our results are in contrast with conclusions of recent LAPW (*18*) and pseudopotential (*19*) calculations for $MgSiO_3$ compositions that orthorhombic perovskite (*Pbnm*) is thermodynamically favored over cubic and tetragonal structures for the entire pressure range of the lower mantle. In both of these studies, the free energy of the perovskite structure is particularly sensitive to the rotations of the SiO_6 octahedra. We infer that the substitution and site occupancy of iron in perovskite may alter the energetics of these distortions and thereby stabilize the high-symmetry phase. Because these reactions appear to involve a finite change in volume, they would require a significant change in compositional models of the lower mantle if they are geologically possible within Earth.

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8. For each synchrotron run, we compressed a single crystal of a natural bronzite in a NaCl medium in a diamond anvil cell. The NaCl served as an infrared window for the CO_2 laser and as a thermal insulator between the sample and the diamond anvil. The crystals were polished to a thickness of ~7 μm to minimize the vertical temperature gradients. Micropositioning motors allowed alignment of the x-ray beam with a spatial resolution of ±1 μm within the sample [J. Hu, H. K. Mao, J. F. Shu, R. J. Hemley, in *High Pressure Science and Technology*, S. C. Schmidt, Ed. (American Institute of Physics, Washington, DC, 1994), pp. 441–444]. Because the CO_2 laser is able to heat the entire crystal and we visually observed the sample throughout the experiment, we were assured that x-ray diffraction was obtained from the laser-heated region. All diffraction measurements were carried out at the smallest scattering angle (2θ) that was feasible for our optical system (12°) to allow maximum dispersion in energy of the diffraction spectra. For the spectroradiometric measurements, the laser-heated spot was imaged through an aperture onto the spectrograph. We determined the temperatures by fitting the blackbody spectra to Wien's law [D. Heinz and R. Jeanloz, in *High Pressure Research in Mineral Physics*, M. H. Manghnani and Y. Syono, Eds. (Terra Scientific, Tokyo, and American Geophysical Union, Washington, DC, 1987), pp. 113–127]. Because we imaged the entire laser-heated region, we determined an average temperature over the heated portion of the sample.
9. Electron microprobe analysis shows that the composition of our sample by mole fraction is SiO_2, 49.65%; MgO, 40.67%; FeO, 6.39%; CaO, 1.73%; and Al_2O_3, 1.54%.
10. The pressures and their uncertainties are derived from room-temperature volume measurements across the sample. Elastic models of samples in the laser-heated diamond cell indicate that the thermal pressure for perovskite at temperatures above 1300 K may be 3 to 5 GPa [D. L. Heinz, *Geophys. Res. Lett.* **17**, 1161 (1990)].
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ILR1, an Amidohydrolase That Releases Active Indole-3-Acetic Acid from Conjugates

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In plants, the growth regulator indole-3-acetic acid (IAA) is found both free and conjugated to a variety of amino acids, peptides, and carbohydrates. IAA conjugated to leucine has effects in *Arabidopsis thaliana* similar to those of free IAA. The *ilr1* mutant is insensitive to exogenous IAA-Leu and was used to positionally clone the *Arabidopsis ILR1* gene. *ILR1* encodes a 48-kilodalton protein that cleaves IAA–amino acid conjugates in vitro and is homologous to bacterial amidohydrolase enzymes. DNA sequences similar to that of *ILR1* are found in other plant species.

IAA, the most widespread and abundant auxin, is a signaling molecule that modulates division and elongation of plant cells. IAA regulates developmental events including embryo symmetry establishment (*1*), root initiation, and apical dominance, as well as environmental responses such as gravitropism and phototropism (*2*). Most IAA in plants is found conjugated through its carboxyl group to a variety of amino acids, peptides, and carbohydrates (*3*). These conjugates represent ~95% of the IAA pool, and they are postulated to inactivate excess IAA, allow rapid alteration of free IAA concentration, and transport IAA through the plant (*3*). Although conjugation and deconjugation are likely control mechanisms in the modulation of free IAA concentrations during development and growth, the enzymes involved in these processes are only beginning to be characterized. The maize *iaglu* gene, which encodes an enzyme that esterifies IAA to glucose, has recently been cloned (*4*), and enzymes that hydrolyze IAA-glucose (*5*) or IAA-Ala have been partially purified (*6*). To determine the role of IAA conjugates in vivo, we isolated *Arabidopsis* mutants with defects in deconjugation.

More than 80% of the IAA pool in *Arabidopsis* is in an amide-linked form (*7*), but

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these conjugates have not been structurally characterized in detail. We tested several IAA–amino acid conjugates to identify those with IAA-like effects on *Arabidopsis* growth. Seedlings germinated on 3 μM IAA had shortened primary roots and increased induction of lateral roots. Several IAA–amino acid conjugates (IAA-Ala, IAA-Gly, IAA-Phe, and IAA-Leu) mimicked IAA effects (Fig. 1) (*8*), whereas others (IAA-Asp, IAA-Ile, and IAA-Val) did not (*8*). These results suggest either that *Arabidopsis* can hydrolyze certain IAA conjugates to release free IAA or that some conjugates themselves have hormone activity.

To distinguish between these alternatives, we screened for mutants insensitive to IAA-Leu (*9*), with the expectation that some of these mutants would be unable to cleave, transport, or import IAA–amino acid conjugates. Mutant plants with increased primary root growth on IAA-Leu were allowed to self-fertilize, and seeds from the next generation were tested for resistance to IAA and IAA-Leu. Some mutants were resistant to both compounds, and these may represent alleles of the IAA-resistant mutants *aux1*, *axr1*, and *axr2* (*10*). We also found four mutants that were resistant to IAA-Leu but were not resistant to free IAA (Fig. 1). These mutants were recessive and defined a single complementation group, *ilr1*.

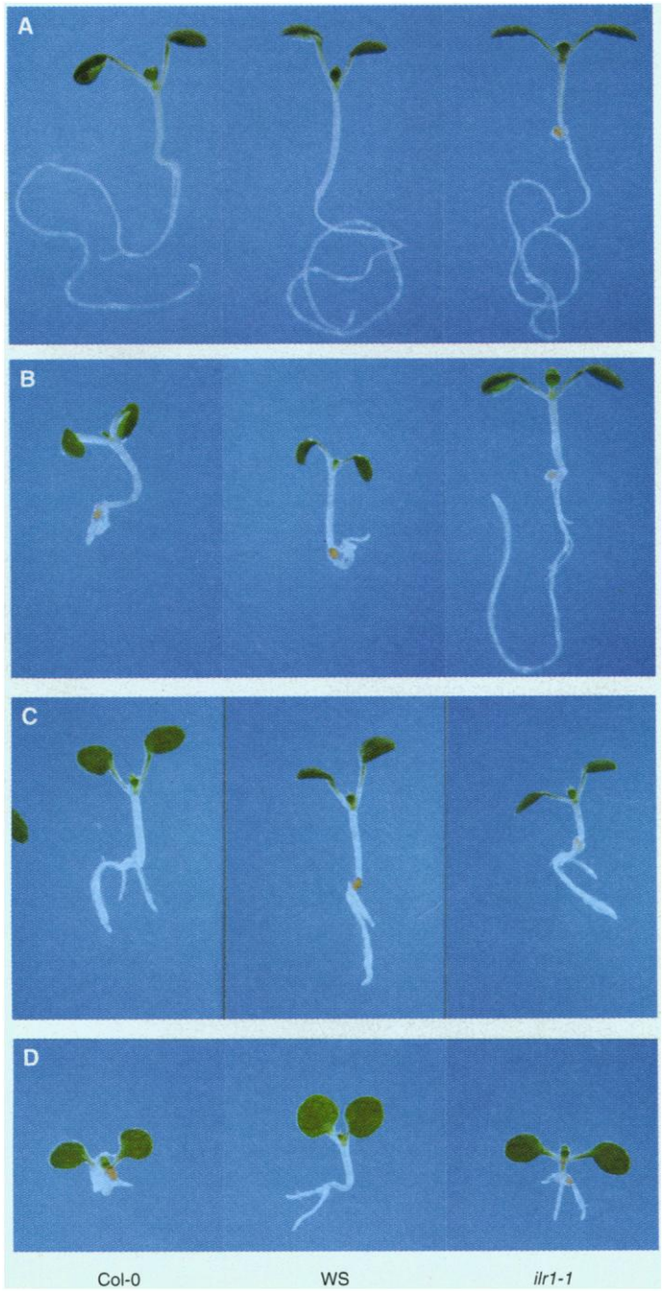

Fig. 1. The *ilr1* mutant is resistant to IAA-Leu, but not to free IAA or IAA-Ala. Seedlings were grown for 9 days on minimal medium with sucrose (0.5%) (*30*) (**A**) or on the same medium supplemented with 50 μM IAA-Leu (**B**), 50 μM IAA-Ala (**C**), or 3 μM IAA (**D**). The mutants shown are homozygous F_3 plants from the third outcross of *ilr1-1* (isolated in the ecotype WS) to the ecotype Col-0.

We cloned the *ILR1* gene by means of a map-based positional approach. The *ilr1* alleles (isolated in the ecotype WS) were crossed to two mapping ecotypes, Col and Ler. DNA prepared from the resultant *ilr1*/*ilr1* F_2 plants was analyzed by the polymerase chain reaction (PCR) at loci polymorphic for length (*11*) or for restriction enzyme sites (*12*). The *ilr1* mutation was localized between nga32 and nga172, two polymorphic markers on chromosome 3 that were separated by less than 2 centimorgans (cM) (Fig. 2A) (*11*). Yeast artificial chromosomes (YACs) containing *Arabidopsis* genomic DNA surrounding these markers had previously been identified (*13*), and we isolated the ends of insert DNA (*14*) from these YACs to orient them into a contiguous stretch of DNA spanning the *ILR1* locus (Fig. 2B). We developed new polymorphic markers (*12*) from the YAC insert ends and used these markers to refine the *ILR1* position to a ~0.5-cM region between the left end of yUP17D8 and the right end of yUP4G12 (*15*). The 60-kb segment between these YAC ends was cloned from a λ-based *Arabidopsis* genomic library (*16*), and the region containing *ILR1* was further narrowed to ~30 kb by analysis of an additional polymorphic marker made from DNA in the interval (Fig. 2C) (*17*).

We identified expressed sequences in the *ILR1* region by probing an *Arabidopsis* complementary DNA (cDNA) library (*18*) with subclones from the λ clones. Seven classes of cDNAs were isolated, and their inferred positions and the sizes of the longest cDNAs isolated for each are shown in Fig. 2D. We sequenced portions of the cDNAs (*19*), and comparison of these sequences to the GenBank database (release 86.0) revealed that the protein encoded by cDNA A (Fig. 2D) was similar (Fig. 3) to bacterial aminoacylase (*20*) and hippuricase (*21*). These en-

Table 1. Substrate specificity of the ILR1 enzyme expressed in *E. coli*. Rates shown for the Phe, Leu, and Ala conjugates are the mean ± SD of three time points (*23*). Hydrolysis of the other substrates was not measurable at the early time points, and rates were calculated from 3-hour reactions. Extracts prepared from *E. coli* transformed with control vector did not hydrolyze any substrate at detectable concentrations (<10 pmol min^{-1} mg^{-1} protein).

Substrate	ILR1 activity (IAA released, pmol min^{-1} mg^{-1} protein)
IAA-Phe	1600 ± 100
IAA-Leu	1200 ± 160
IAA-Ala	200 ± 70
IAA-Gly	100
IAA-Val	92
IAA-Ile	<10
IAA-1-O-β-D-glucose	<10
IAA-*myo*-inositol	<10

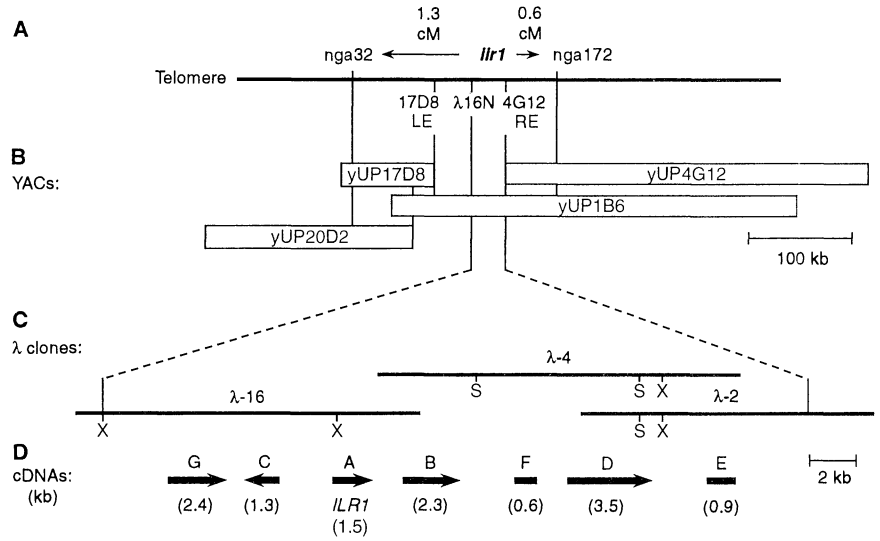

Fig. 2. Genetic and physical map of the genomic region containing *ILR1*. (**A**) Analysis of a population segregating for *ILR1* and *ilr1* localized *ILR1* to the region of chromosome 3 bounded by the polymorphic markers nga32 and nga172 (*11*). (**B**) Alignment of YAC clones spanning the *ILR1* locus. End probes were isolated from the YACs hybridizing to nga32 or nga172 (*13*) by plasmid rescue (*14*). Vertical lines represent probes used for hybridization or detection of polymorphisms. (**C**) λ clones spanning the *ILR1* locus were isolated by hybridization (*16*). Sal I (S) and Xho I (X) restriction sites are indicated. (**D**) Seven expressed sequences encoded entirely within the λ clones shown in (C) were isolated by hybridization. Arrowheads indicate the direction of transcription, when known.

zymes both cleave amides, as would an enzyme that hydrolyzes IAA-Leu (Fig. 4).

To test whether cDNA A was *ILR1*, we used PCR to amplify genomic DNA from each of the four *ilr1* alleles and directly sequenced these PCR products (*19*). Each of three alleles (*ilr1-1*, *ilr1-2*, and *ilr1-3*) generated by ethyl methanesulfonate (EMS) contained a single base pair difference (which resulted in an amino acid substitution) from the gene in the unmutagenized parental strain (Fig. 3). The allele *ilr1-4* (*22*) contained a single base pair deletion at codon 400, which resulted in a frame shift at this position. These differences, along with the fact that the mutations in *ilr1-1* and *ilr1-2* changed amino acids that were identical among cDNA A and the bacterial amidohydrolases (Fig. 3), support the conclusion that cDNA A encodes the *ILR1* gene.

To determine whether ILR1 catalyzed IAA–amino acid hydrolysis, we expressed the *ILR1* cDNA in *Escherichia coli* and tested extracts from the resultant strain for conjugate cleavage activity (*23*). Extracts from cells expressing *ILR1* cleaved IAA-Leu to yield free IAA (Table 1), whereas extracts from control strains transformed with the vector did not. The partially purified IAA-Ala hydrolase from carrot requires Mn^{2+} for maximal activity (*6*), and the bacterial aminoacylase from *Bacillus stearothermophilus* requires Co^{2+} (*20*). Both Mn^{2+} and Co^{2+} enhanced ILR1 activity, whereas EDTA decreased ILR1 activity (*8*). ILR1 cleaved both IAA-Leu and IAA-Phe with similar efficiency, and it cleaved IAA-Ala, IAA-Gly, and IAA-Val with low efficiency (Table 1). IAA-Ile, which lacks IAA-like effects in *Arabidopsis* (*8*), was not hydrolyzed by ILR1 nor were the ester conjugates of IAA with glucose or inositol. The in vitro substrate specificity of the ILR1 enzyme mirrored the in vivo resistance of the *ilr1* mutant, which was slightly resistant to IAA-Phe (*8*) as well as to IAA-Leu (Fig. 1) but remained sensitive to IAA-Ala (Fig. 1) and IAA-Gly (*8*). These findings suggest that the resistance of the *ilr1* mutant to IAA-Leu and IAA-Phe is the result of decreased cleavage of these conjugates rather than an import defect. The isolation of mutants that are resistant to both IAA-Ala and IAA-Gly but not to IAA-Leu or IAA-Phe (*8*) suggests that IAA–amino acid conjugates fall into at least two classes in *Arabidopsis*. Whether these classes serve overlapping or distinct functions during growth and development remains to be determined.

ILR1 is a member of a small gene family. A partially sequenced *Arabidopsis* cDNA

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ILR1  MDFSGSFFVIFVTFFFLPPLSSAGSYDSGSGLESLARGMLHSAKDPEEFEWMRGIRRKIHENPETGFQEFKTSQL  75
ILL1  MALNNFL--TFQLLLLLLRVSSESPWIVAGDVSRIPINFLELAKSPEVFDSMVRIRRKIHENPELGYEEFETSKF  73
ILL2  MALNKLLSLTFQLLLFLLSVSSESPWI-AEDTSQIQTKLLEFAKSPEVFDWMVKIRRKIHENPELGYEELETSKL  74
hipp                                    MNLIPEILDLQGEFE---KIRHQIHENPELGFDELCTAKL  37
acyl                                  MTKEEIKRLVDEVKTDVIAW----RRHLHAHPELSFQEEKTAQF  40

ILR1  VRDELDSLGVKYKYPVAKTGVVAWIGSCSKPV-FGLRADMDALPLQELVEWESKSKVDGKMHACGHDTHVAMLLG  149
ILL1  IRSELDLIGVKYRFPVAITGIIGYIGTGEPPF-VALRADMDALPIQEAVEWEHKSKNPGKMHACGHDGHVAMLLG  147
ILL2  IRSELELIGIKYRYPVAITGVIGYIGTGEPPF-VALRADMDALPIQEGVEWEHKSKIPGKMHACGHDGHVTMLLG  148
hipp  VAQKLKEFGYEVYEEIGKTGVVGVLKKGNSDKKIGLRADMDALPLQECTNLPYKSKKENVMHACGHDGHTTSLLL  112
acyl  VYETLQSFGHLELSRPTKTSVMARLIGQQFGRVVAIRADMDALPIQEENTFEFASKNPGVMHACGHDGHTAMLLG  115

ILR1  AAKLLQTTKHLIKGTVKLVFQPGEEGY-AGAYEMLKDEILDDLDG--ILSVHVFPSIPSGGIGSRPGTVLAGAGL  221
ILL1  AAKILQQHRQHLQGTVVLIFQPAEEGL-SGAKAMREEGALKNVEA--IFGIHLSPRTPFGKAASLAGSFMAGAGA  219
ILL2  AAKILHEHRHHLQGTVVLIFQPAEEGL-SGAKAMREEGALKNVEA--IFGIHLSARIPFGKAASRAGSFLAGAGV  220
hipp  AAKYLA--SQNFNGTLNLLYFQPAEEGL-GGAKAMIEDGLFEKFDSDYVFGWHNMPFGSDKKFYLKKGAMMASSDS  184
acyl  TAKIFSQLRDDIRGEIRFLFQHAEELFPGGAEEMVQAGVMDGVDV--VIGTHLWSPLERGKIGIVYGPMMAAPDR  188

ILR1  FTVTVHGQGSHAATPHFSKDPVLAASSAVVALQQIVSRELDPLEAGVVTVGYIEGGHAQNVIPQSAKFGGTFRSL  296
ILL1  FEAVITGKGGHAAIPQHTIDPVVAASSIVLSLQHLVSRETDPSDSKVVTVTKVNGGNAFNVIPDSITIGGTLRAF  294
ILL2  FEAVITGKGGHAAIPHHTIDPVVAASSIVLSLQQLVSRETDPLDSKVVTVSKVNGGNAFNVIPDSITIGGTLRAF  295
hipp  YSIEVIGRGGHGSAPEKAKDPIYAASLLVVALQSIVSRNVDPQNSAVVSIGAFNAGHAFNIIPDIVTIKMSVRAL  259
acyl  FFIRTIGKGGHGAMPHQTIDAIAIGAQVVTNLQHIVSRYVDPLEPLVLSVTQFVAGTAHNVLPGEVEIQGTVRTF  263

ILR1  SNDGLLFIQRRIKEISEAQASVYRCKAEVNFEEKKPSLHPVMNNDEGLYEHGKKVAEAMIGKNNFH-DFPVTMGG  370
ILL1  T--GFTQLQERIKEIITKQAAVHRCNASVNLAPNGNQPMPPTVNNMDLYKKFKKVVRDLLGQEAFV-EAVPEMGS  366
ILL2  T--GFTQLQQRVKEVITKQAAVHRCNASVNLTPNGREPMPPTVNNKDLYKQFKKVVRDLLGQEAFV-EAAPVMGS  367
hipp  DNETRKLTEEKIYKICKGLAQAN----DIEIKINKNVVAPVIMNNDEAVDFASEVAKELFGEKNCEFNHRPLMAS  330
acyl  DETLRRTVPQWMERIVKGITEAH--GASYEFRFD-YGYRPVINYDEGDPRHGGNGVRAAVRRRGSGPLE-------  329

ILR1  EDFSFFTQKTKAAIFVLGIKNETLGAGKPLHSPYFFVDEEALPVGAALHAAMAVSYLDEHG-HSHEEEVKSEL  442
ILL1  EDFSYFAETIPGHFSLLGMQDETQGYASS-HSPHYRINEDVLPYGAAIHATMAVQYLKDKASKGSVSGFHDEL  438
ILL2  EDFSYFAETIPGHFSLLGMQDEINGYASS-HSPLYRINEDVLPYGAAIHASMAVQYLKEKASKGSVSGFHEEL  439
hipp  EDFGFFCEMKKCAYAFLENENDIY-----LHNSSYVFNDKLLARAASYYAKLALKYLK                  383
acyl  ------TEHGRRRFLRLFAKSARQLFLR--RRGQCRKRHRLPAPPPALYD                         370
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Fig. 3. Alignments of the deduced amino acid sequences (*25*) of the predicted ILR1 (cDNA A), ILL1, and ILL2 proteins of *Arabidopsis* with the hippuricase enzyme (hipp) from *Campylobacter jejuni* (*21*) and an aminoacylase (acyl) from *Bacillus stearothermophilus* (*20*). Sequences were aligned with the program Megalign (DNAStar) using the Clustal method (*33*). Amino acid residues identical in at least three of the five sequences are boxed, and hyphens indicate gaps introduced to maximize alignment. The mutations in the four *ilr1* alleles are $Gly^{139} \rightarrow Asp^{139}$ (*ilr1-1*), $Gly^{415} \rightarrow Glu^{415}$ (*ilr1-2*), $Glu^{69} \rightarrow Lys^{69}$ (*ilr1-3*), and a single base pair deletion at codon 400 (*ilr1-4*). Nucleotide sequences were deposited in GenBank (accession numbers: *ILR1*, U23794; *ILL1*, U23795; and *ILL2*, U23796).

(GenBank accession number T13869) showed similarity to *ILR1*. We used this partial cDNA (*24*) to probe an *Arabidopsis* cDNA library (*18*) by hybridization and isolated two classes of cDNAs: the *ILR1*-like gene (*ILL1*), used as a probe, and a second *ILR1*-related gene (*ILL2*). We sequenced these two cDNAs (*19*), and their deduced amino acid sequences are about 87% identical to one another, 43% identical to ILR1, and 30% identical to the bacterial hydrolases (Fig. 3).

ILR1 and *ILL1* potentially encode 48-kD proteins that are similar along their lengths to the bacterial amidohydrolases, with the exception of a hydrophilic COOH-terminus and a stretch of hydrophobic amino acids at the NH_2-terminus with characteristics of a signal sequence (Fig. 3). ILL1 has a sequence at its COOH-terminus, HDEL (*25*), that has been shown to be essential for active retrieval of proteins into the lumen of the endoplasmic reticulum (ER) (*26*), which suggests that some IAA conjugate hydrolases function in the ER. The major auxin-binding protein in maize, ABP1, also has an ER retrieval signal (*26*), consistent with a role for IAA metabolism in this compartment.

DNA sequences similar to *ILR1* were detected by low-stringency hybridization of the *ILR1* cDNA to DNA isolated from a variety of other dicots, including tomato, carrot, and bean (*8*). In addition, comparison of the ILR1 sequence to the DNA databases uncovered several partial sequences from anonymous rice cDNAs that potentially encode sequences similar to the ILR1 protein (*27*). These results suggest that IAA–amino acid hydrolases function in all flowering plants and that hormone deconjugation is a general control mechanism in IAA metabolism.

A correlation has been observed between the hydrolysis of exogenously applied IAA–amino acid conjugates and their biological activity (*28*). Our results strongly suggest that certain conjugates are cleaved in vivo and that this hydrolysis is necessary for IAA activity. Elucidating the regulation and localization of ILR1 and related IAA–amino acid hydrolases may reveal the roles of their corresponding conjugates during development. Ultimately, the ability to control deconjugation may allow the manipulation of IAA concentrations in vivo to influence the size and shape of plants.

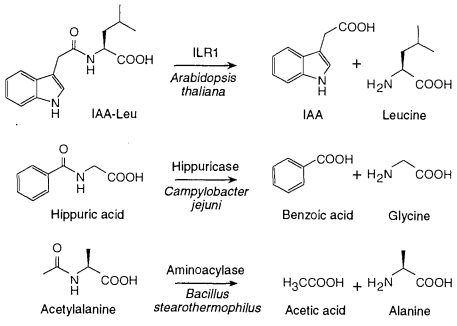

Fig. 4. Reactions catalyzed by ILR1, hippuricase (*21*), and aminoacylase (*20*).

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15. Genetic mapping used segregating populations from crosses between *ILR1/ILR1* (ecotypes Col-0 or Ler) and *ilr1/ilr1* (ecotype WS) plants. Genomic DNA was prepared (J. Celenza and G. R. Fink, in preparation) from about 700 *ilr1/ilr1* F_2 plants and scored with the polymorphic markers 17D8LE and 4G12RE (*17*).
16. The *Arabidopsis* λ genomic library was provided by J. Mulligan and R. Davis. For each step in the cloning process, a gel-purified DNA fragment probe was used to isolate a set of overlapping λ clones by hybridization (*32*). These clones were characterized by restriction mapping and Southern (DNA) blot analysis (*32*), and a new DNA fragment from one of the isolated clones was chosen for use as a subsequent probe.
17. To score polymorphic markers, we amplified genomic DNA by PCR, cleaved the resultant fragments with restriction enzymes, and detected polymorphisms by agarose gel electrophoresis (*32*). The oligonucleotides and enzymes used were 17D8LE (5′-CTCCTTTGTCATCTCCCGAATC-3′ and 5′-CCAACAACATGCATGATAGTTCAG-3′), Hinc II; 4G12RE (5′-CAAACAAAATCAAATGACGG-3′ and 5′-CCCGAACCAATTCAATGATTC-3′), Dde I; and λ16N (5′-GCGTCTTCGAGTAACTTTATC-3′ and 5′-GCGGGAAACGGAGAATTTGAAAC-3′), Afl II.
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19. Complementary DNAs were sequenced with oligonucleotide primers by means of alkaline denaturation of double-stranded templates (*32*). Because no full-length cDNAs were obtained for *ILL1*, the first three amino acids were deduced from the sequencing of a genomic clone (*8*). The transcribed region of the *ILR1* gene was amplified by PCR (with the use of the oligonucleotides 5′-CCGTTGTCCTTTCTTTCATTGACA-3′ and 5′-GAAAGTGGTTGGTTTCTCGTGACT-3′) from genomic DNA prepared from WS (wild type) and from each of the four *ilr1* mutant alleles. The amplified fragments were sequenced directly (*32*), and nucleotide differences were verified from independently generated PCR products. Reported sequences were determined on both strands.
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22. The *ilr1-4* allele was recovered from pools of seeds descended from transgenic plants carrying insertions of foreign DNA (*29*). The mutation that caused the IAA-Leu^R phenotype in this strain was not linked to the inserted DNA (*8*) and may be spontaneous in origin.
23. The *ILR1* cDNA was cloned into the *E. coli* expression vector pET15b (Novagen) as a gene fusion from codon 23. The *E. coli* strain BL21 [DE3]/pLysS [F. W. Studier, A. H. Rosenberg, J. J. Dunn, J. W. Dubendorff, *Methods Enzymol.* **185**, 60 (1990)] was used for expression. Cells were grown in 75 ml of LB medium (*32*) supplemented with carbenicillin (100 μg/ml) and chloramphenicol (25 μg/ml) at 36°C to an optical density at 600 nm (OD_{600}) of ~0.1; isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 1 mM, and growth was continued for 3 hours. Cells were harvested by centrifugation, resuspended in 2 ml of lysis buffer (200 mM Hepes, 2 mM dithiothreitol, and 0.05% Triton X-100, pH 7.0), and lysed by freezing at –70°C followed by thawing at 25°C. Viscosity was reduced by passage through a 26G syringe, and extracts were added to two volumes of lysis buffer containing 1 mM substrate (IAA-Leu, and so forth) and 1.5 mM $MnCl_2$. Reactions were allowed to proceed at 25°C for 0.5, 1.5, or 3 hours and were terminated by addition of two volumes of 50% ethanol and 1% acetic acid. Samples were clarified by centrifugation and analyzed by high-performance liquid chromatography with a Zorbax C-18 column (4.6 by 250 mm, Dupont) at 2 ml/min. The solvent system was a gradient of 15 to 30% methanol (20 min) followed by a gradient of 30 to 99% methanol (15 min) with 1% acetic acid throughout. Compounds were detected at 278 nm and were quantitated by peak area. Protein concentrations were determined by the dye-binding method with the use of a kit from Bio-Rad (Melville, NY).
24. From the *Arabidopsis* Biological Resource Center, Ohio State University.
25. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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27. For example, the rice clone RICR0601A is 56% identical to ILR1 over 128 amino acid residues.
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